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Education Ministry Guidelines on Recombinant DNA Experiments

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Guidelines From the Ministry of Education on Recombinant DNA Experiments in Universities

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[Text]

Education Ministry Notification No. 4

The guidelines for conducting recombinant DNA experiments at universities and other research institutes, covered by the 1982 Education Ministry Notification No. 131, will be revised as contained herein.

31 January 1991
Education Minister Yutaka Inoue

Chapter 1. General Rules

1. Purpose

These guidelines cover the standards that are required to be observed by universities and other research institutes in conducting recombinant DNA experiments, and are aimed at ensuring experiment safety and promoting the experiment.

2. Definitions

The following are definitions of the terms and phrases appearing in these guidelines.

1) "Recombinant DNA experiment" refers to an experiment involving the production of recombination molecules between a DNA (vector), which is capable of multiplying within a living cell, and a hetero-DNA inside a test tube, and the promotion of multiplication of a hetero-DNA by importing the molecules into the intended living cells. The phrase also refers to an experiment involving the use of the recombinant obtained through such an experiment.

2) "Host" denotes the living cells into which recombination DNA molecules are imported.

3) "Vector" refers to a living cell that is capable of multiplying within the host and that transports a hetero-DNA into the host.

4) "Host-vector system" refers to a combination of a host and a vector.

5) "Recombination DNA molecule" refers to a cluster of molecules consisting of a vector and a hetero-DNA molecule.

6) "Recombinant" refers to a living cell into which recombination DNA molecules were imported.

7) "DNA donor" refers to a living cell or a microorganism that offers the DNA to be inserted into the vector. In the case of inserting DNA synthesized using RNA as a template, the cell or microorganism offering the RNA is also called a DNA donor.

8) A recombinant DNA experiment involves a) an "experiment for a recombinant creation" or b) an "experiment for recombinant multiplication."

a) "Experiment for a recombinant creation" refers to an experiment for obtaining a recombinant bearing an unidentified hetero-DNA and to an experiment involving the use of such recombinant.

b) "Experiment for recombinant multiplication" refers to an experiment for multiplying a recombinant bearing a specific, identified DNA and to an experiment involving the use of a multiplied recombinant. A multiplication experiment also includes an experiment that involves a change in the gene structure in the identified-DNA recombinant or in a host-vector system.

9) "Universities and other institutes" refers to universities, technical colleges, research institutes open to university researchers, and establishments that are under the jurisdiction of the education minister or other academic and research establishments that were founded under provisions in Civil Law Article 34 (Meiji 29 Law No. 89) under the supervision of the minister.

3. Guideline Application Range

These guidelines are designed to cover recombinant DNA experiments to be conducted at universities and other research institutes.

Chapter 2. Basic Considerations for Securing Experiment Safety

Section 1. General Rules

1. Basic Consideration for Securing Experiment Safety

Basically, recombinant DNA experiments must be conducted in compliance with standard safety procedures taken in ordinary experiments involving the use of microorganisms. When circumstances require, both physical confinement measures and biological confinement measures must be taken to ensure safety.

2. Purpose of Confinement

1) Physical confinement is aimed at preventing the unwanted exposure of researchers and other people to the recombinant and its leakage out of the experiment zone by confining it within the experiment equipment or the lab.

2) Biological confinement is aimed at preventing escape of a recombinant into the atmosphere by using a specific host-vector system.

3. Procedure for Securing Experiment Safety

Because of the importance of securing experiment safety, all recombinant DNA experiments are required to go

through the safety procedures stipulated in these guidelines. Recombinant DNA experiments can be classified, by type of procedures, into the following four categories.

a) An experiment that requires permission from the education minister before conducting the experiment.

b) An experiment that requires permission from the head of an academic or research institute.

c) An experiment in which submission of an experiment plan to the head of an academic or research institute is required before conducting the experiment.

d) An experiment that requires none of the above.

Section 2. Evaluation of Experiment Safety

1. Basic Consideration for Experiment Safety Evaluation

1) Experiment for Creating a Recombinant

a) In a recombinant DNA experiment, an evaluation of the biological safety of DNA donors and host-vector systems to be used in the experiment must be conducted.

b) In an experiment involving the use of a DNA donor having a gene that is able to produce a protein toxic to vertebrate animals, a safety evaluation of the toxicity must be conducted in addition to the safety evaluation described above.

2) Experiment for Recombinant Multiplication

a) In a recombinant multiplication experiment, an evaluation must be conducted of the biological safety of host-vector systems and the DNA to be introduced into the host using a vector.

b) In an experiment involving the use of a DNA that is able to produce a protein toxic to vertebrate animals, an additional safety evaluation, as described in 1-b), must be conducted.

c) When a recombinant is cultured in volume, an additional safety evaluation may become necessary in addition to the evaluations described in a) and b) above.

d) When an experiment is conducted outside the lab, an additional safety evaluation must be conducted in addition to the evaluations described in a) and b) above by taking into consideration the environmental conditions in and around the place where the experiment is to be conducted.

Section 3. Safe Preservation and Transport of Recombinant Samples and Wastes

1. Basic Consideration for Preservation and Transport of Recombinant Samples and Wastes

For the sake of safety, preservation of recombinant samples and wastes, as well as their transportation, must be properly made.

Chapter 3. Education, Training, and Health Management of Research Personnel

1. Education and Training

Before the start of an experiment, the heads of the universities and the faculties concerned and those at the academic and research institutes responsible for overseeing an experiment are required to familiarize research personnel with the guidelines and internal safety regulations that were drawn based on the guidelines. In addition, they are also required to give safety education and training to researchers.

2. Health Management

1) Heads of the universities, the faculties, and the other research institutes are required to give regular medical examinations to those engaged in recombinant DNA experiments and to introduce other health-keeping measures by seeking advice from the safety committee set up within these establishments.

2) These heads are required to update the examination records and preserve them.

3) Persons engaged in an experiment are required to pay close attention to their health, and when they notice any health problem, they must report it to the heads of their establishments.

Chapter 4. Organization for Ensuring Experiment Safety

1. Heads of Universities and Other Research Institutes

1) Heads of universities and other research institutes are responsible for securing experiment safety and are required to make efforts toward this end.

2) In addition to the obligations described in Chapter 3, these heads are required to:

a) Choose the members of the safety committee and the officials devoted to safety preservation for overseeing the implementation of experiment safety measures.

b) After a safety committee screening, submit an application to the education minister for an experiment requiring government permission.

c) After a safety committee screening, give an approval for an experiment requiring approval of the heads.

d) Accept an experiment plan, which is required to be submitted to the heads.

e) Work out an internal safety regulation through deliberations by the safety committee.

f) Implement other experiment safety-related programs.

2. Safety Committee

1) Universities and other research institutes are required to set up a safety committee within their establishments.

2) The safety committee must be made up of experts, considering that the committee is required to deal with highly specialized matters.

3) The committee is required to deliberate on the following issues and submit a report or give advice on the issues to the heads of the universities and other research institutes.

a) Establishment or abolition of an internal safety regulation.

b) Suitability of an experiment plan under the guidelines and the internal safety regulation.

c) Education and training of research personnel and their health management.

d) Measures to deal with an accident when it occurs, and the measures for preventing it.

e) Other vital issues.

4) The safety committee can ask the chief of the experiment or the officials devoted to safety preservation to submit a report when the need arises.

3. Officials Devoted to Safety Preservation

1) Universities and other research institutes are required to have officials devoted to safety preservation; these officials will assist the heads of the establishments on safety matters.

2) The officials are required to be well-versed in the guidelines and the internal safety regulations and are required to be knowledgeable about biological accidents as well as being well-experienced in dealing with such accidents. They are required to:

a) Keep watch over whether a given experiment is being conducted in accordance with the guidelines and the internal safety regulations.

b) Give proper advice and guidance to the chief of the experiment.

c) Implement the necessary measures.

3) The safety officials, in implementing their task, are required to maintain good communication with the safety committee and, when the need arises, to submit a report to the committee.

4. Chiefs of Experiments

1) Each experiment team at universities and other research institutes is required to have a chief who takes overall responsibility for the experiment.

2) The chiefs are required to be familiar with the guidelines and the internal safety regulations and to have good knowledge and skills in preventing biological accidents from occurring. They are required to implement the following tasks in addition to giving education and training to the researchers working under them, as described in Chapter 3-1.

a) In planning and implementing an experiment, the chiefs are required to oversee the experiment by respecting the guidelines and the internal safety regulations and by maintaining good communication with the safety officials.

b) In conducting an experiment requiring government approval, they are required to submit the experiment plan to the heads of the universities and other research institutes. When a change is made with an already-submitted plan, they are required to submit a document notifying of the change.

c) In conducting an experiment requiring approval by the head of the university or research institute, the chiefs are required to submit an experiment plan to the head to obtain approval. When a change is made with the plan, they are also required to obtain an approval.

d) In conducting an experiment requiring approval by the head of a research establishment, the chief is required to submit an experiment plan to the head. The same is required when there is a change in the already-submitted plan.

e) The chiefs are also required to carry out other tasks that may arise during an experiment.

5. Researchers

Researchers involved in a recombinant DNA experiment are required to understand the importance of maintaining safety when conducting the experiment, and are required to have good knowledge and experience in handling microorganisms, about experiment methods to be used and manipulation operations involved, and in other related technical matters.

Chapter 5. Securing Safety in Quasirecombinant DNA Experiments

In conducting a quasirecombinant DNA experiment, the guidelines are applied to ensure experiment safety where the guidelines refer to such an experiment.

Chapter 6. Miscellaneous Regulations

The guidelines will also be applied to recombinant DNA experiments conducted by the research organizations, excluding the universities and other research institutes referred to so far, when the experiments are being conducted by receiving government subsidies for promoting science research. In this case, the experiments described in Chapter 5 are also included.

Appendix

Contents

Appendix Section 1. Physical Confinement Facilities, Design of Lab, and Required Experiment Practices (A matter related to Chapter 2, Section 1, 2-1))

I. Physical Confinement in Recombinant DNA Production Experiment and the Multiplication Experiment (Excluding Experiments Involving the Use of a Culture Medium Volume in Excess of 20 Liters)

The physical confinement levels range from P1 to P4. Details concerning confinement facilities, design of lab, and required experiment practices in each of these levels are as follows.

1. P1 Level

1) Confinement Facilities and Design of Lab

The experiment lab is required to have facilities as good as in an ordinary microorganism experiment lab.

2) Required Experiment Practices

a) The doors and windows of the lab must be closed during an experiment.

b) Experiment tables must be sterilized every day after finishing the experiment. When contamination occurs during an experiment, sterilization measure should be taken immediately.

c) The wastes, including recombinant DNA samples, must be sterilized before disposal. Contaminated equipment must be washed before using it again, or it must be either sterilized or disinfected before disposal.

d) It is advisable to use a mechanical pipet.

e) Eating, smoking, and storing food inside the lab should be avoided.

f) Hands should be well washed after handling a recombinant DNA sample or before leaving the lab.

g) Care should be exercised to minimize the generation of aerosol in every lab operation.

h) When sterilization of a contaminated object is conducted outside the lab, the object must be hauled out of the lab by being contained within a sturdy, leak-proof container.

i) The lab must be kept free of insects and rodents.

j) Use of a syringe should be avoided when other methods are available.

k) Researchers should wear a white lab uniform when they engage in an experiment, and take it off when they leave the lab.

l) The inside of the lab should always be kept clean and tidy.

m) Researchers are required to comply with any safety-related decisions made by the chief of the experiment.

2. P2 Level

1) Confinement Facilities

When using a blender, a freeze dryer, an ultrasonic cell crusher, or a centrifugal separator—machines that can generate unwanted aerosol easily in processing recombinant DNA samples—proper measures should be taken to prevent escape of contaminated aerosol out of lab. In using these machines, it is advisable to use a safety cabinet (see Appendix Table 1). The effectiveness of using the cabinet should be checked whenever the need arises.

2) Design of Lab

A lab should be constructed inside a building equipped with a high-pressure sterilizer for treating contaminated objects and experiment wastes.

3) Required Experiment Practices

a) The doors and windows of the lab should be kept shut.

b) The experiment tables and the safety cabinets should be sterilized every day after the day's experiment is finished. When a contamination occurs during an experiment, sterilization steps should be taken immediately.

c) All experiment wastes, including recombinant DNA samples, must be sterilized before disposal. Contaminated equipment should be washed before using again and should be sterilized before disposal.

d) A mechanical pipet should be used.

e) Eating, smoking, and storing food in the lab should be avoided.

f) Before leaving the lab after handling recombinant DNA samples, hands should be washed.

g) Care should be taken to minimize the generation of aerosol in the lab.

h) When sterilizing a contaminated object outside the lab, the object must be hauled out by being contained within a sturdy, leakproof container.

i) Insects and rodents should be eliminated from the lab.

j) Use of a syringe should be avoided when other methods are available.

k) A white lab uniform should be worn at all times while in the lab, and when leaving the lab it should be taken off.

l) No person who knows little about the experiment under way must be allowed to enter the lab alone.

m) When an experiment is being conducted, a sign announcing that a P2-level experiment is under way inside should be posted at the lab entrance.

n) The lab should always be kept clean and tidy.

o) When a P1-level experiment is conducted in parallel with a P3-level experiment in a P3 lab, a clear borderline should be maintained between the two separate areas where these experiments are conducted, and the experiments should be conducted with the utmost care.

p) Researchers must comply with any procedural decisions made by the chief of the experiment.

3. P3 Level

1) Confinement Facilities

a) In conducting a recombinant DNA experiment, safety cabinets capable of accommodating aerosol-producing machines should be installed in the lab. However, the use of such a safety cabinet is not necessary when the aerosol-producing machine is designed to be capable of containing the generated aerosol inside the machine unit.

b) In installing a safety cabinet, it should be placed in a way that allows regular checking, replacement of the HEPA filter, and fumigation to be conducted without moving the cabinet. Immediately after installation, checks on the following three points should be made, and a regular check on items i) and ii) should be implemented more than once a year.

i) Wind speed and air capacity test.

ii) HEPA filter test.

iii) Sealing capability test.

2) Design of Lab

a) The lab is required to have an experiment zone consisting of a "doorway" room for controlling entrance and exit of people, corridors, and experiment rooms isolated from nonexperiment quarters, by the corridors and the doorway room. The room should be equipped with doors at the front and in the rear, which do not open simultaneously, and a clothes-changing room.

b) A high-pressure sterilizer for sterilization of contaminated objects and experiment wastes should be installed in the experiment zone.

c) The floors, walls, and ceilings in the experiment zone should be of a structure and covered with a material that allows washing the surface and fumigating the zone easily.

d) At the main doorway in the experiment zone or the lab, a hand washing facility that can be activated with an elbow, a foot, or automatically should be installed.

e) The windows in the experiment zone should be kept shut.

f) A vacuum suction system in the experiment zone should be installed independently from ones in other quarters in the lab building, and it should be used exclusively for recombinant DNA experiments. The suction inlet should be capped with a filter or a sterilizer trap.

g) In the experiment zone, an air treatment system should be installed such that air flows in a direction from the doorway room into the zone. In the system, the circulating air in the experiment zone should be exhausted outside after filtration and additional treatment.

3) Required Experiment Practices

a) Throughout the experiment, the door in the lab should be kept shut.

b) The experiment tables and the safety cabinets must be sterilized every day after the day's experiment is finished. When a contamination occurs during the experiment, the contaminated object must be sterilized immediately.

c) All the experiment wastes, including recombinant DNA samples, must be sterilized before disposal. Contaminated equipment must be washed before using it again, and when it is to be disposed it must be sterilized.

d) A mechanical pipet should be used.

e) Eating, smoking, and storing food in the experiment zone should be avoided.

f) Hands should be washed when leaving the experiment zone after handling recombinant samples.

g) Care should be exercised to minimize the generation of aerosol in the experiment zone.

h) When sterilizing a contaminated object outside the experiment zone, the object must be hauled out and transported by being contained in a sturdy, leakproof container.

i) Insects and rodents should be eliminated from the experiment zone.

j) Use of a syringe should be avoided as much as possible where other methods are available.

k) In the experiment zone, researchers should put on a long-sleeved buttonless experiment uniform that can be worn by slipping the body into it head first. When leaving the zone, the uniform should be taken off. When the robe is laundered, it should be sterilized.

l) Entrance to the experiment zone must be made through the doorway room, and no person having little knowledge about the on-going experiment must be permitted to enter the zone alone.

m) When an experiment is under way, a sign announcing that a P3-level experiment is now being conducted should be posted at both the entrance to the experiment zone and to the lab.

n) The experiment zone must always be kept clean and tidy, and nothing having little to do with the experiment should be left in the zone.

o) In handling a recombinant DNA sample, experiment gloves must be worn. After finishing work using the gloves, they must be taken off by taking care that they do not contaminate anything else, and the gloves must then be sterilized.

p) When a P3-level experiment is going on, no other experiments belonging to the P1 or P2 confinement levels must be conducted in parallel.

q) Researchers in the experiment zone must comply with the procedural decisions made by the chief of the experiment.

4. P4 Level

1) Confinement Facilities

a) Class III safety cabinets for handling recombinant DNA samples must be installed. However, when the experiment is conducted at the special zone within the experiment zone, either class I or II cabinets can be used in place of class III cabinets when the researchers inside are doing an experiment wearing a positive-pressured coverall-type protective uniform within a ventilated safety cabinet equipped with a life-support system. The special experiment zone denotes an experiment space within an airtight compartment equipped with a life-support system, created within the experiment zone.

b) The safety cabinet must be installed in a way such that, without moving it, the cabinet's regular check, change of HEPA filter, and fumigation with formaldehyde can be conducted. Immediately after installation, the safety cabinet must be checked on the following three points, and a regular check on items i) and ii) must be carried out more than once every year.

i) Air speed and air volume test (excluding class III cabinet).

ii) HEPA filter capability test.

iii) Airtightness test.

2) Design of Lab

a) Access to the experiment zone by people who are not involved in a recombinant DNA experiment must be limited by building either a lab building devoted to recombinant DNA experiments or a building in which the experiment quarter is clearly isolated from nonexperiment quarters.

b) The doorway room leading to the experiment zone must be equipped with doors at the front and in the rear that do not open simultaneously and with a clothes-changing room and a shower room.

c) When a researcher is required to bring a sample or other experiment-use objects into the experiment zone without the clothes-changing room procedure, he must be made to walk through a gateway compartment equipped with an ultraviolet-ray radiation facility, in which the doors at the front and in the rear are designed not to open simultaneously.

d) The lab must have floor, walls, and ceiling structure that allows easy conduct of a washing and a fumigation inside the room, prevents invasion of insects and rodents, and can maintain a vaporized sterilizing chemical at a constant pressure for a period. However, this does not necessarily mean that the room must be airtight.

e) In the lab or the experiment zone, a hand-washing facility that can be activated by an elbow, a foot, or automatically must be installed.

f) The doors in the experiment zone must be of an automatically opening and closing mechanism.

g) The main vacuum suction facility for use in the experiment zone must be built independently from ones in other experiment quarters, and HEPA filters should be installed as close to the vacuum power utilization points and the checking cocks as possible. The filter must be installed in a way that allows its sterilization and replacement to be conducted easily.

h) The pipes supplying gas and water to the experiment zone must be equipped with a mechanism preventing reverse flow.

i) The lab must have a walk-through high-pressure sterilization compartment, in which the doors at the both ends do not open simultaneously, to sterilize anything hauled out from the experiment zone.

j) To sterilize experiment samples and other things that cannot be sterilized by using heat, for hauling out from the experiment zone, a sterilization chemical-filled tub or a walk-through fumigation compartment, in which the doors at the both ends do not open simultaneously, must be installed.

k) The experiment zone ventilation system that is independent from other labs must be installed. The system must be so designed that air taken in from outdoors flows toward the experiment zones of increasing danger, and the system must be equipped with a mechanism for preventing reverse air flow. The system must also be equipped with a warning system indicating a malfunction or other system troubles.

l) Air circulated in each lab must be filtered using HEPA filters.

m) Air from the experiment zone must be filtered by an HEPA filter before being exhausted outdoors, and the exhaust must be done at a place as far away from a nearby building and the air intake of a nearby building as possible. The HEPA filter must be installed in a way that easily allows its sterilization, its replacement, and the conduct of a capability test.

n) The treated air from class III safety cabinets must be exhausted outdoors. However, the treated air from class I and II safety cabinets can be exhausted into the lab. When the treated air from these cabinets is exhausted through the ventilation system installed exclusively for the experiment zone, the exhaust must be arranged in a way that does not disturb the balance in air circulation in the ventilation system in the experiment zone.

o) The special experiment zone set up within the experiment zone is required to satisfy the following requirements.

i) The life-support system must be equipped with an emergency air tank and an alarm.

ii) The doors in the special experiment zone must be airtight.

iii) A chemical shower room for sterilizing contaminated clothes must be installed.

iv) The ventilation air from the special experiment zone must be filtered twice, using HEPA filters, before being exhausted outdoors.

v) As a safety precaution, two separate ventilation systems must be installed.

vi) In preparation for an emergency, the special experiment zone must be equipped with an auxiliary power generator, lighting equipment, and a communication system.

vii) Air pressure in the special experiment zone must be maintained at a level lower than other experiment quarters around the zone.

viii) A high-pressure sterilizer must be installed in the special experiment zone to sterilize experiment wastes before their being disposed outside the zone.

3) Required Experiment Practices

a) When an experiment is being conducted, the doors and windows in the lab must be kept shut.

b) The experiment tables and the safety cabinets must be sterilized every day after the day's experiment is finished. When a contamination occurs during an experiment, a sterilization must be made immediately.

c) All the wastes from the lab, including recombinant DNA samples, must be sterilized before disposal. Contaminated equipment must be washed before being used again, and it must be sterilized before disposal.

d) A mechanical pipet must be used.

e) Eating, smoking, and storing food in the special experiment zone must be avoided.

f) Hands must be washed when leaving the zone after handling a recombinant DNA sample.

g) Generation of aerosol must be minimized in every operation conducted in the zone.

h) Removing a living biological sample from the experiment zone or from a class III safety cabinet in the zone must be done by putting the sample into a sturdy, airtight container and then dipping the container once in a sterilizer solution-filled tank or fumigating it for sterilization. A similar procedure must be taken when removing a living biological sample into the class III cabinet in the experiment zone.

i) To remove a recombinant DNA sample or equipment from the special experiment zone or the class III safety cabinet in the zone, excluding the case described in h), the sample or equipment must be sterilized using a high-pressure sterilizer. When there is a possibility that subjecting the sample or equipment to the high pressure sterilization could lead to damage to the thing to be sterilized, because of the high-temperature vapor involved, they must be either dipped in a sterilization solution-filled tank or put into fumigation. A similar step must be taken when bringing anything into the class III safety cabinet in the special experiment zone.

j) Insects and rodents must be eliminated from the zone.

k) When other means are available, the use of a syringe should be avoided as much as possible.

l) No persons other than those involved in the experiment must be admitted into the special experiment zone.

m) Entrance into the experiment zone must be made through the doorway room, and a shower must be taken when entering and leaving the zone.

n) When entering the special experiment zone, a proper form of protective uniform, made up of a combination of underwear, a pair of trousers, shirts, an upper garment, a pair of shoes, a hood, and a pair of gloves, must be worn. When leaving the experiment zone, the uniform must be taken off and put into the designated box and a shower must be taken.

o) A sticker carrying the internationally-used logo denoting biological hazard must be put on all doors, freezers, and refrigerators that store recombinant DNA samples in the special experiment zone.

p) The experiment zone must always be kept clean and tidy, and anything having nothing to do with the on-going experiment must be kept out of the zone.

q) The inside of the safety cabinets must be fumigated with 10 g/m^3 formaldehyde by keeping the cabinet doors closed shortly before the replacement of the HEPA filters or when a regular check of the safety cabinet is conducted.

r) Used fluids from the safety cabinets and lab sinks must be heat-treated for sterilization. Used water from the shower room and the hand-washing facility must be sterilized chemically.

s) When a P4-level experiment is being conducted, another experiment at the P3 level or lower must not be conducted simultaneously in the special experiment zone.

t) Researchers must comply with other experiment procedural decisions made by the chief of the experiment.

II. Physical Confinement in Experiments Involving Volume-Cultured Recombinant Samples

The physical confinement levels consist of LS-C level, LS-1 level, and LS-2 level, and the requirements in designing and maintaining the confinement facilities and the required experiment practice at each of these levels are as follows.

1. LS-C Level

1) Confinement Facilities and Their Design

a) Culturing facility and other experiment facilities and equipment must always be kept in good condition.

b) A recombinant culturing facility must be designed to minimize the recombinant being cultured escaping out of the culturing chamber when the chamber gas is exhausted.

2) Required Experiment Practices

a) Wastes and waste fluid originating from an experiment using a recombinant must be sterilized and purified before they are disposed. The effectiveness of the treatment must be ascertained against the host to be used in the experiment before starting the experiment.

b) When planting a recombinant in the culturing equipment, or when sampling a cultured recombinant for use in an experiment from the equipment, contamination of the outer surface of the equipment must be minimized.

c) When moving a recombinant from the culturing equipment to other experiment equipment or containers, care must be taken to minimize contamination as a result of leak of the recombinant.

d) An experiment zone that handles a large volume of recombinant within the lab must always be kept clean, and insects and rodents must be eliminated from the zone.

e) A sign must be posted on culturing equipment and other experiment machines used in an LS-C level experiment involving a large volume of cultured recombinant, indicating that they are being used for an experiment.

f) Concerning the proper form of protective clothes to be worn during experiment, researchers are required to comply with the judgments made by the chief of the experiment.

g) Researchers must comply with other experiment procedural decisions made by the chief of the experiment.

2. LS-1 Level

1) Confinement Facilities and Their Design

a) The recombinant culturing equipment to be used in an LS-1 level experiment is required to have a design that prevents leak of recombinant fluid and allows sterilization of the culturing chamber to be carried out without opening the door. An airtightness test of the equipment must be conducted immediately after it is installed and, after that, regularly once a year.

b) When using machines that can easily generate unwanted aerosol in processing a recombinant, such as a blender, a freeze dryer, an ultrasonic cell crusher, or a centrifugal separator, the machines must be used inside an airtight safety cabinet or a similar other aerosol-confinement unit having the equivalent confinement capability. These safety cabinets must be checked for airtightness immediately after their installation and, after that, regularly once a year.

c) The recombinant culturing equipment must be designed so that exhaust gas from the culturing chamber is exhausted through a filter or other additional unit having an equivalent capability to eliminate microorganisms in the exhaust gas. These filters must be checked for efficiency immediately after their installation and, after that, regularly once a year.

d) Every time a modification or a replacement of parts is made at a place vital in maintaining good confinement condition in an equipment or a machine, an airtightness test must be conducted immediately after the modification or replacement.

2) Required Experiment Practices

a) An experiment zone involving the use of a large volume of cultured recombinant must be clearly defined.

b) Culturing equipment and other contaminated experiment machines must be sterilized after the day's experiment is finished, and the wastes and waste fluids originating from the recombinant used in an experiment must be sterilized before disposal. The effectiveness of the sterilization must be ascertained against the host to be used in the experiment before starting the experiment.

c) The use of a mechanical pipet is preferred. When manipulating a pipet by mouth, a pipet having a cotton plug must be used.

d) Eating, smoking, and storing food in an experiment zone involving the use of a large volume of recombinant must be avoided.

e) Hands must be washed after handling a recombinant or when leaving the experiment zone after finishing work there.

f) In every manipulation conducted in the zone, care must be taken to minimize the generation of unwanted aerosol.

g) Planting a recombinant in the culturing equipment, or sampling a cultured recombinant from the equipment for use in an experiment, must be done by taking care that the outside surface of the equipment is not contaminated. When a contamination occurs, the equipment must be sterilized immediately.

h) When moving a recombinant from the culturing equipment to other equipment or containers, care

must be exercised not to let the recombinant solution leak out of the equipment or containers. When a contamination occurs, sterilization must be made immediately.

i) Except in the case of working inside a safety cabinet, or in the cases described in g) and h), the fluid containing a recombinant must not be removed from the culturing equipment before the fluid is sterilized. The effectiveness of the sterilization must be ascertained against the host to be used in the experiment before starting the experiment.

j) When sterilizing a contaminated item outside the designated experiment zone, the item must be hauled out by being contained in a sturdy, leakproof container.

k) Insects and rodents must be eliminated from an experiment zone involving the use of a large volume of recombinant.

l) When an LS-1 level experiment involving the use of a large quantity of a recombinant is being conducted, a sign indicating that such an experiment is under way must be posted at an appropriate place in the experiment zone.

m) Concerning the proper form of protective clothes, the researchers are required to comply with the judgments made by the chief of the experiment.

n) When an experiment is under way, the container of cultured recombinant must be checked more than once every day for its airtightness and other safety precautions.

o) The filters in the safety cabinets or in other similar installations for removing microorganisms from the exhausted air must be sterilized when they are replaced and when a regular check of the cabinets or the installations is conducted.

p) When a P1-level experiment is simultaneously conducted in an LS-1 level experiment zone, it must be carefully conducted by limiting activities within the defined P1 experiment zone.

q) The researchers must comply with other experiment procedural decisions made by the chief of the experiment.

3. LS-2 Level

1) Confinement Facilities and Their Design

a) The culturing equipment to be installed in an LS-2 level experiment zone must be designed to prevent leak of a recombinant from being cultured and allow sterilization of the equipment's culturing chamber to be carried out by keeping the door closed. Extra care must be taken in designing the rotating seal, pipe valves, and other parts directly connected to the equipment to prevent leak of the recombinant inside the equipment. The culturing equipment must be checked for airtightness immediately after its installation and every time it is used in an experiment.

b) When using a blender, freeze dryer, ultrasonic cell crusher, or centrifugal separator that can easily generate unwanted aerosol when treating a recombinant, the machines must be used inside a class II safety cabinet or other similar installation having an equivalent confinement capability. These confinement installations are not needed when the machines are equipped with an aerosol-confinement design.

c) The exhaust gas from the culturing equipment chamber must be exhausted through a microorganism-removal filter having a capability equivalent to or better than that of HEPA filters, or through other removal units having a similar capability. These filtering facilities must be checked for their capability immediately after installation and, after that, once a year during a regular check.

d) A class II safety cabinet and other similar installations should be installed in a way that, without moving them, allows a regular check of these installations, replacement of microorganism-removal filters and other similar removal units, and formaldehyde fumigation of the installations. These installations must be checked for the following three check items immediately after installation and, after that, a regular check for items i) and ii) must be made at least once a year.

i) Air speed and air volume test.

ii) Capability of microorganism-removal filters and other similar removal units.

iii) Airtightness test.

e) An indicator for monitoring airtightness throughout an experiment must be installed on the culturing equipment, on the units connected to the equipment directly, on the safety cabinets, and on other similar confinement installations.

f) All the equipment and facilities used in an experiment must be put under strict management by putting stickers carrying a series of identification numbers and by recording the numbers in every relevant experiment document, including inspection records and equipment manipulation records.

g) The lab must be created inside a building equipped with a high-pressure sterilizer for sterilizing experiment wastes and contaminated objects.

h) Every time that a modification or a replacement of parts has been made to a confinement section in the equipment or facilities, the tests for airtightness and other capabilities must be conducted immediately after the modification or replacement.

2) Required Experiment Practices

a) During an experiment, the windows in the lab must be kept shut. Opening and closing of lab door must be kept to a minimum.

b) The culturing equipment and other lab equipment and facilities contaminated during an experiment must be sterilized after the day's experiment is over. Wastes and waste fluids originating from a recombinant experiment must be sterilized before disposal. The effectiveness of the sterilization must be ascertained against the host to be used in the experiment before starting the experiment.

c) A mechanical pipet must be used.

d) Eating, smoking, and storing food in the lab must be avoided.

e) Hands must be washed after handling a recombinant or when leaving the lab.

f) In every manipulation of equipment and machines, care must be exercised to minimize the generation of aerosol.

g) Sampling a recombinant from the culturing equipment for use in an experiment must be done carefully so as not to contaminate the outer surface of the equipment. When a contamination occurs, sterilization must be made immediately.

h) When moving a recombinant from culturing equipment to other equipment or machines, care must be exercised to prevent a leak. When a contamination occurs, sterilization must be made immediately.

i) Culturing solution containing a recombinant must not be taken out of the culturing equipment or other confined places before the solution is sterilized. This does not apply when the action takes place inside a class II safety cabinet or other confinement installation, and in the cases of g) and h) above. The effectiveness of sterilization must be ascertained against the host to be used in the experiment before starting the experiment.

j) Sterilizing a contaminated object outside the lab must be done by hauling out the object within a sturdy, airtight container.

k) Insects and rodents must be eliminated from the lab.

l) When entering the lab, a protective uniform must be put on, and when leaving the lab, the uniform must be taken off.

m) Persons having no knowledge about the experiment under way must not be admitted into the lab.

n) When an experiment is being conducted, a sign announcing that an LS-2 level experiment involving the use of a large quantity of a recombinant is under way inside must be posted at the lab entrance.

o) The lab must always be kept clean and tidy, and no objects unrelated to the experiment being conducted must be kept in the lab.

p) During an experiment, the operating conditions of the culturing equipment, the confinement units connected to the equipment directly, and the class II safety cabinets being used must be monitored constantly through a confinement condition monitoring system.

q) The microorganism-removal filters and other filtering units installed in class II safety cabinets and other similar confinement installations in the lab must be sterilized by fumigating inside these installations with a 10 g/m^3 formaldehyde gas for about one hour and keeping the doors shut immediately before replacement of the filters, or when a regular check of their capability is conducted, or when there is a change in experiment plan.

r) When P1 or P2 level experiments or LS-C or LS-1 level experiments are simultaneously conducted within the LS-2 level experiment lab, the experiments must be confined strictly within separately set experiment zones.

s) Researchers must comply with other experiment procedural decisions made by the chief of the experiment.

Appendix Section 2. Levels of Biological Confinement (A matter related to Chapter 2, Section 1, 2-2)

1. Biological Confinement

1) When a procaryote or a lower eucaryote are used as a host in an experiment, their biological confinement levels are either B1 or B2, depending on the safety levels of the host-vector system. To which of the confinement levels a given host-vector system belongs is decided by the education minister based on standards worked out by the Education Ministry's Science Council (see Appendix Table 2).

2) When cultured animal or plant cells are used as a host, their biological confinement level is B1. When the safety level is significantly high, the confinement level can be changed to B2 with permission of the education minister. When using an infectious virus as a host, a different set of confinement levels is applied.

2. Biological Confinement Levels

1) B1 Level

This biological confinement level is applied to a host-vector system in which the host has a low survival capability under natural environment conditions and the vector has a high dependency for its survival on the host and cannot easily be accommodated by other types of

cells, thus making it relatively easy to prevent the system-based recombinant escaping into the atmosphere. The same confinement level is also applied to a host-vector system that has a comparatively high biological safety to human beings, judging from its genetic and physiological characteristics and the ecological behavior in natural environment.

2) B2 Level

This confinement level is applied to a host-vector system in which the host has a particularly low survival capability under natural environment conditions and the vector has a particularly high dependency on the host for its survival, thus making it possible to prevent the system-based recombinant's escape into the environment.

3. [Host-Vector System Particulars]

Before permission is granted by the education minister on a matter related to a host-vector system, judgment must be made on the following matters.

1) The merit of adopting a given host-vector system.

2) The genetic character of the biological species to which the host-vector system intended to be used belongs.

a) Biological behavior under natural environment condition.

b) Physiological characteristics.

c) Range of genetic exchange and the exchange mechanism.

d) Pathogenicity and toxic substance production capability.

e) Parasitic and saprobic characteristics.

f) History of a contact with human beings.

g) Easiness of sterilization in lab.

3) The genetic character of a host-vector system to be used.

a) Origin and hysteresis.

b) Range of genetic exchange.

c) Dependency of a vector on the host.

4) When trying to obtain permission for a B2 level experiment, information about the process of producing a host and a vector, the reason for adopting the variation, the character of the variation, and the method for adopting the host-vector system must be supplied, in addition to the information about a) to g) described in 2) above.

5) The reason that a host-vector system to be used belongs to either B1 or B2 level based on the results of a judgment on above-mentioned items in a) to g) described in 2) above.

4. Host-Vector Systems and Their Biological Confinement Levels

Appendix Table 2 gives the host-vector systems having permission for use by the education minister and their biological confinement levels.

Appendix Section 3. Safety Evaluation for Recombinant DNA Creation Experiment and the Application Procedure (A matter related to Chapter 2, Section 2, 1-1)-a))

Concerning the DNA donors used in an experiment, a safety evaluation must first be made about 1) *procaryote* (including rickettia and chlamydia) and *lower eucaryote* (including autosynthetic factors (virus included)), 2) *eucaryote* virus and viroid (excluding ones belonging to lower eucaryote), and 3) eucaryote (animals and plants).

1. Classification of Safety Levels of DNA Donors Used in an Experiment

Among the DNA donors that can be used in recombinant DNA experiment, classification of the safety levels of procaryote and lower eucaryote is given in Appendix Table 3, and that of eucaryote virus and viroid in Appendix Table 4.

2. Classification by Procedure Involved in an Experiment for Creation of a Recombinant (see Table A [at the end of this Appendix Section])

1) The classifications by type of experiment in the creation of a recombinant using a permitted host-vector system and by procedure involved in an experiment conducted using a permitted host-vector system are as follows.

a) Experiment requiring education minister's permission:

i) An experiment involving the use of a procaryote or a lower eucaryote as a DNA donor that has been found to have a pathogenicity.

ii) An experiment involving the use of any of the eucaryote viruses listed in Appendix Table 4-(1) or any of those that are not included in Appendix Table 4 as a DNA donor.

iii) An experiment that does not belong in the following items b), c), and d).

b) Experiments requiring research establishment's permission:

i) An experiment involving the use of any of the procaryote or the lower eucaryote listed in Appendix Table 3-(1) as a DNA donor (confinement level: B1/P3 or B2/P2).

ii) An experiment involving the use of any of the procaryote or the lower eucaryote listed in Appendix Table 3-(2) as a DNA donor (confinement level: B1/P2 or B2/P1).

iii) An experiment involving the use of any of the eucaryote viruses listed in Appendix Table 4-(2) as a DNA donor (confinement level: B1/P3 or B2/P2).

iv) An experiment involving the use of any of the eucaryote viruses listed in Appendix Table 4-(3) as a DNA donor (confinement level: B1/P2 or B2/P1).

v) An experiment involving the use of an animal, excluding lower eucaryote, as a DNA donor (confinement level: B1/P2 or B2/P1).

c) Experiment requiring research establishment's acknowledgment:

i) An experiment involving the use of any of the procaryote or the lower eucaryote that are not included in Appendix Table 3, excluding one that has been found to have a pathogenicity, as a DNA donor (confinement level: P1).

ii) An experiment involving the use of any of the eucaryote viruses or the viroids that are listed in Appendix Table 4-(4) as a DNA donor (confinement level: P1).

iii) An experiment involving the use of a plant, excluding ones belonging to lower eucaryote, as a DNA donor (confinement level: P1).

d) Experiment requiring no permission or acknowledgment:

An experiment for creating a recombinant using different types of organisms, among which it is believed that there is a genetic exchange under natural environment conditions.

2) Categorization based on preparation required for an experiment for creating a recombinant using a host-vector system.

a) Experiment requiring education minister's permission: An experiment that does not belong to the following b) and c).

b) Experiment requiring research establishment's permission: An experiment involving the use of any of the host-vector systems included in Appendix Table 5 as a DNA donor.

c) Experiment requiring none of these permissions: An experiment for creating a recombinant using different types of organisms, among which it is believed that there is a genetic exchange under natural environment conditions.

The following table summarizes that has been described so far in this section.

Table A. Classification of Safety Levels of DNA Donors by Type of Host-Vector System

	DNA donors	Physical confinement levels to be combined with the biological confinement levels used in the experiment	
		Biological confinement level B1	Biological confinement level B2
The cases of using permitted host-vector system			
	(1) Among *procaryote* (including rickettia and chlamydia) and *lower eucaryote* (including autosynthesis factors (virus included) originating from it), those that have been found to have a pathogenicity. (The definitions for the italicized terms also apply to the same terms appearing in the following.)	Experiment requiring education minister's permission	
	(2) Among procaryote and lower eucaryote, those listed in Appendix Table 3-(1)	Experiment requiring research establishment's permission: P3	Experiment requiring research establishment's permission: P2
	(3) Among procaryote and lower eucaryote, those listed in Appendix Table 3-(2)	Experiment requiring research establishment's permission: P2	Experiment requiring research establishment's permission: P1
	(4) Among procaryote and lower eucaryote, those that are not listed in Appendix Table 3, excluding ones belonging to 3	Experiment requiring research establishment's acknowledgment: P1	Experiment requiring research establishment's acknowledgment: P1
	(5) Among *eucaryote* virus (excluding ones belonging to lower eucaryote) those that are not listed in Appendix Table 4. (The definition for the italicized term applies to the same term appearing in the following.)	Experiment requiring education minister's permission	
	(6) Among eucaryote virus, those listed in Appendix Table 4-(1)	Experiment requiring education minister's permission	

Table A. Classification of Safety Levels of DNA Donors by Type of Host-Vector System (Continued)

	DNA donors	Physical confinement levels to be combined with the biological confinement levels used in the experiment	
	(7) Among eucaryote virus, those listed in Appendix Table 4-(2)	Experiment requiring research establishment's permission: P3	Experiment requiring research establishment's permission: P2
	(8) Among eucaryote virus, those listed in Appendix Table 4-(3)	Experiment requiring research establishment's permission: P2	Experiment requiring research establishment's permission: P1
	(9) Among eucaryote virus and viroid, those listed in Appendix Table 4-(4)	Experiment requiring research establishment's acknowledgment: P1	Experiment requiring research establishment's acknowledgment: P1
	(10) Animals (excluding those belonging to lower eucaryote)	Experiment requiring research establishment's permission: P2	Experiment requiring research establishment's permission: P1
	(11) Plants (excluding those belonging to lower eucaryote)	Experiment requiring research establishment's acknowledgment: P1	Experiment requiring research establishment's acknowledgment: P1
	(12) An experiment for creating a recombinant from different species of organisms among which it is believed that an exchange of genes takes place under natural environment conditions	Experiment requiring neither permission nor acknowledgment	
	(13) Experiments belonging to none of those described above	Experiment requiring education minister's permission	
The cases of using unpermitted host-vector systems	(1) An experiment for creating a recombinant between different species of organisms among which it is believed that there is an exchange of genes under natural environment conditions	Experiment requiring neither permission nor acknowledgment	
	(2) An experiment involving the use of any of the DNA donors and the host-vector systems listed in Appendix Table 5	Experiment requiring research establishment's permission	
	(3) Experiments which belong to neither of the above two categories	Experiment requiring education minister's permission	

Note: Concerning the experiment involving the use of a DNA donor carrying a gene having a capability of producing a protein toxic to vertebrate animals, see Appendix Section 4.

Appendix Section 4. Safety Evaluation for Experiments Involving Donors Carrying a Gene Capable of Producing a Protein Toxic to Vertebrate Animals in Recombinant Creation Experiment and the Evaluation Procedure (A matter related to Chapter 2, Section 2, 1-1)-b))

The classification of the application procedures for an experiment involving the use of a DNA donor carrying a gene capable of producing a protein toxic to vertebrate animals when the toxic LD_{50} level is below 100 μg per kilogram of body weight is as follows.

1. Experiment Involving Use of EK1 or EK2 (see Appendix Table 2)

1) Permission from the education minister is required for an experiment handling a DNA donor having an LD_{50} level lower than 100 ng per kilogram of the body weight.

2) Permission from the research establishment is required for an experiment handling an LD_{50} level higher than 100 ng per kilogram of body weight and lower than 100 μg per kilogram. (Confinement level: B1/P2 or B2/P1)

2. Experiment Using [Donors] Other Than EK1 and EK2

Permission from the education minister is required for all experiments involving the use of those donors.

Appendix Section 5. Safety Evaluation for Recombinant Multiplication Experiments and Application Procedure (A matter related to Chapter 2, Section 2, 1-2)-a))

The classification of application procedures for an experiment in which the volume of the recombinant to be cultured does not exceed 20 liters is as follows (see Table B at the end of this Appendix Section).

The biological characteristics manifested within a host by a DNA introduced into a vector, which must be evaluated for safety, are: 1) pathogenicity, 2) fixity, parasitic factor, 3) infectiousness, 4) toxic substance production capability, 5) carcinogenic factor, 6) antimedicine capability, 7) capability of producing metabolism disturbing substance, and 8) the nature of causing a disturbance in the ecological system. Overall safety evaluation is made by taking these characteristics into consideration.

When conducting an experiment on recombinant multiplication using a recombinant having a specific DNA or recombinant DNA molecules, which were created at other research establishments, the user establishment is regarded to have conducted the recombinant creation experiment under the said guidelines, and thus the user establishment is required to comply with the application procedural requirements in the experiments described below.

1) Experiment Requiring Education Minister's Permission

The following four experiments fall under the category of experiments requiring the education minister's permission, even when they are conducted as experiments also belonging to the following categories 2), 3), and 4).

a) An experiment involving the multiplication of a recombinant created in an experiment permitted by the education minister, when conducting a recombinant creation experiment by relaxing the physical confinement level by one notch based on the evaluated safety level of the identified recombinant DNA molecules involved in the experiment.

b) An experiment involving the multiplication of a recombinant created in an experiment permitted either by the education minister or a research establishment, when conducting a recombinant creation experiment by relaxing the physical confinement level by two notches based on the evaluated safety level of the identified recombinant DNA molecules involved in the experiment.

c) A recombinant creation experiment involving a change in the host-vector system being used, when conducting an experiment using a procaryote or a lower eucaryote which has been found to have a pathogenicity as the host.

d) A recombinant creation experiment involving a change in the host-vector system used, when conducting an experiment using cultured animal or plant cells as the host and either those viruses listed in Appendix Table 4-(1) or not listed in Appendix Table 4 as the vector. [This includes an experiment using a combination of a defective virus and a helper virus. (This applies to a case of using a virus or viroid as a vector whenever the same reference is made in the following passages.)]

2) Experiment Requiring Research Establishment's Permission

When conducting the following experiments, permission of the research establishment must be obtained before starting the experiment. When conducting an experiment that belongs to more than one item of these safety guideline descriptions, the highest of the confinement levels involved applies.

a) An experiment for multiplying a recombinant that was created in an education minister-permitted experiment, when conducting an experiment that is conducted at the same confinement level as the physical confinement level mandated in creating the recombinant.

b) An experiment for multiplying a recombinant in a research establishment-permitted experiment, when conducting an experiment by relaxing the physical confinement level by one notch based on the results of evaluation of the character of the identified recombinant DNA involved in the experiment.

c) A recombinant creation experiment involving a change in the host-vector system used, when conducting an experiment using a procaryote or a lower eucaryote as the host.

i) An experiment involving the use of any of the procaryote or lower eucaryote listed in Appendix Table 3-(1) as the host. (Confinement level: P3)

ii) An experiment involving the use of any of the procaryote or lower eucaryote listed in Appendix Table 3-(2) as the host. (Confinement level: P2)

iii) An experiment involving the use of any of the procaryote or lower eucaryote not listed in Appendix Table 3 (excluding those that have been found to have a pathogenicity) as the host. However, this excludes an experiment involving the use of an officially permitted host-vector system. (Confinement level: P1)

d) A recombinant creation experiment involving a change in the host-vector system used, when conducting an experiment using cultured animal or plant cells as the host and virus or viroid as the vector. However, when conducting an experiment to produce an infectious virus recombinant using any of the viruses listed in Appendix Table 6 as the vector, the physical confinement level must be set one step higher.

i) An experiment involving the use of any of the viruses listed in Appendix Table 4-(2) as the vector. (Confinement level: P3)

ii) An experiment involving the use of any of the viruses listed in Appendix Table 4-(3) as the vector. (Confinement level: P2; P3 when using the viruses listed in Appendix Table 6)

iii) An experiment involving the use of any of the viruses or viroids listed in Appendix Table

4-(4) as the vector. (Confinement level: P1; P2 when using any of the viruses listed in Appendix Table 6)

3) Experiment Requiring Research Establishment's Permission

In a recombinant multiplication experiment using a recombinant created in an experiment either permitted or acknowledged by a research establishment, when conducting an experiment at the same confinement level as the physical confinement level mandated when the recombinant was created.

4) Experiment Requiring No Permission or Acknowledgment

An experiment for multiplying a recombinant between different species of organisms among which it is believed that there is an exchange of genes under natural environment conditions.

Table B summarizes what has been described so far in this section. When a recombinant multiplication experiment belongs to more than one item of the experiments listed in the table, the severest of the procedural requirements and the highest of the physical confinement levels involved apply.

Table B. Classification of Safety Levels by Procedural Requirement Needed in Conducting a Recombinant Multiplication Experiment

	Experiment types	Physical confinement levels
The cases of conducting a recombinant creation experiment by easing the physical confinement level	(1) In a recombinant multiplication experiment using a recombinant created in an education minister-permitted experiment, when a recombinant creation experiment is conducted by lowering the physical confinement level by one notch	Experiment requiring education minister's permission
	(2) In a recombinant multiplication experiment using a recombinant created in an education minister- or research establishment-permitted experiment, when a recombinant creation experiment is conducted by relaxing the physical confinement level by two notches	Experiment requiring education minister's permission
	(3) In a recombinant multiplication experiment using a recombinant created in a research establishment-permitted experiment, when conducting a recombinant creation experiment by relaxing the physical confinement level by one notch	Experiment requiring research establishment's permission
The cases of conducting an experiment at the same physical confinement level as one mandated in a recombinant creation experiment	(4) In a recombinant multiplication experiment using a recombinant created in an education minister-permitted experiment, when conducting an experiment at the same physical confinement level as one mandated in the experiment for creating the recombinant	Experiment requiring research establishment's permission
	(5) In a recombinant multiplication experiment using a recombinant created in an experiment permitted or acknowledged by a research establishment, when conducting an experiment at the same physical confinement level as one mandated in the experiment for creating the recombinant	Experiment requiring research establishment's acknowledgment
An experiment involving a change in host-vector system in a recombinant creation experiment	(6) An experiment using a procaryote or a lower eucaryote that has been found to have a pathogenicity as the host	Experiment requiring education minister's permission
	(7) An experiment using any of the procaryote and lower eucaryote not listed in Appendix Table 3 (excluding those that have been found to have a pathogenicity) as the host. (However, this excludes an experiment using officially permitted host-vector systems.)	Experiment requiring research establishment's permission: P1
	(8) An experiment using any of the procaryote and lower eucaryote listed in Appendix Table 3-(1) as the host	Experiment requiring research establishment's permission: P3
	(9) An experiment using any of the procaryote and lower eucaryote listed in Appendix Table 3-(2) as the host	Experiment requiring research establishment's permission: P2
	(10) An experiment using cultured animal or plant cells as the host and any of the viruses not listed in Appendix Table 4 as the vector	Experiment requiring education minister's permission
	(11) An experiment using cultured animal or plant cells as the host and any of the viruses listed in Appendix Table 4-(1) as the vector	Experiment requiring education minister's permission
	(12) An experiment using cultured animal or plant cells as the host and any of the viruses listed in Appendix Table 4-(2) as the vector	Experiment requiring research establishment's permission: P3
	(13) An experiment using cultured animal or plant cells as the host and any of the viruses listed in Appendix Table 4-(3) as the vector. (An experiment using any of the viruses listed in Appendix Table 6 as the vector.)	Experiment requiring research establishment's permission: P2 (P3)

Table B. Classification of Safety Levels by Procedural Requirement Needed in Conducting a Recombinant Multiplication Experiment (Continued)

	Experiment types	Physical confinement levels
	(14) An experiment using cultured animal or plant cells as the host and any of the viruses and viroids listed in Appendix Table 4-(4) as the vector. (An experiment using any of the viruses listed in Appendix Table 6 as the vector.)	Experiment requiring research establishment's permission: P1 (P2)
Other types of experiment	(15) An experiment for multiplication of a recombinant using different species of organisms among which it is believed there is an exchange of genes under natural environment conditions	Experiment requiring none of these permissions and acknowledgment

Note: Concerning the experiment for multiplication of a recombinant carrying DNA having a capability of producing a protein toxic to vertebrate animals, see Appendix Section 6.

Appendix Section 6. Safety Evaluation for Experiments Involving the Use of a DNA Carrying a Gene Capable of Producing a Protein Toxic to Vertebrate Animals in the Experiment When the Volume of Culture Medium Is No More Than 20 Liters and the Evaluation Procedure (A matter related to Chapter 2, Section 2, 1-2)-b))

In a recombinant multiplication experiment involving the use of culture recombinant no larger than 20 liters in volume, when conducting an experiment using a DNA having a capability of producing a protein toxic to vertebrate animals, the same application procedural requirements as ones required in conducting the recombinant creation experiment are applied.

Appendix Section 7. Safety Evaluation for Experiments Involving a Large Volume of Culture Medium and the Evaluation Procedure (A matter related to Chapter 2, Section 2, 1-2))

1. The requirements for confinement in conducting an experiment involving a large volume of recombinant culturing are as follows.

1) When conducting an experiment for culturing a large volume of a recombinant, which is mandated to be conducted at a physical confinement level of P1 when the recombinant volume involved is smaller than 20 liters, a physical confinement level of LS-1 must be applied. When the mandated confinement level is at P2, LS-2 level must be applied.

2) In an experiment involving a large volume of recombinant culturing, in which the recombinant involved was proved to have a particularly high biological safety, the experiment can be conducted at either of the following two confinement levels by obtaining permission from the education minister.

a) LS-C physical confinement level.

b) A special confinement method other than those confinement levels defined in the Appendix Section 1-II.

2. The classification of volume recombinant culturing experiment by application procedural requirement is as follows.

1) Experiment requiring education minister's permission:

Volume recombinant culturing experiments, excluding ones defined in the following items 2) and 3).

2) Experiment requiring research establishment's permission:

a) An experiment of volume culturing of a recombinant that was created by introducing an identified and safe DNA into an officially permitted host-vector system. (Excluding the volume recombinant culturing experiment mandated to be conducted at the physical confinement level of P3 or P4, when the experiment is conducted with the recombinant volume involved smaller than 20 liters.)

b) An experiment of volume culturing of a recombinant that was created by introducing any of those identified and safe DNA donors listed in Appendix Table 5 or a derivative of those DNA donors having the equivalent safety level into those host-vector systems listed in the table.

3) Experiment requiring no permission:

An experiment for volume culturing of a recombinant created from different species of organisms among which it is believed that there is an exchange of genes under natural environment conditions.

Appendix Section 8. Safety Evaluation for a Recombinant DNA Multiplication Experiment Conducted Outside the Lab and the Evaluation Procedure (A matter related to Chapter 2, Section 2, 1-2)-d))

All experiments to be conducted outside a lab require permission from the education minister before they are conducted. In conducting such experiments, their safety must be ascertained through a lab experience before conducting them outside the lab.

Appendix Section 9. Safety Measures To Be Taken in Preserving and Transporting Recombinant Samples and Recombinant Wastes (A matter related to Chapter 2, Section 3, 1)

The measures that must be taken to ensure safety in preserving and transporting recombinant samples and recombinant wastes are as follows.

1. Preservation

1) Containers having recombinant samples or recombinant wastes must carry a sign announcing the contents, and they must be kept in the lab, experiment zone, or volume culturing experiment zone satisfying the physical confinement level mandated for the experiment handling the type of recombinant involved.

The freezing chamber or refrigerator containing samples or wastes must also carry such a sign.

2) The chief of the experiment must keep records of the stored samples and wastes. However, when the sample and wastes contain a recombinant that calls for application of P2 physical confinement level or lower, the record can be replaced with an experiment record.

2. Transportation

1) Moving a P2 or lower confinement level recombinant sample or the recombinant's wastes out of a lab must be done by containing it in a sturdy, airtight container.

2) Moving a P3 or higher confinement level recombinant sample or the wastes out of a lab or the experiment zone must be done by containing it in a sturdy, airtight container having an additional safety structure to prevent leakage of the contents even if the container is damaged. The container must carry a "Handle With Care" sign written in red letters on the surface where the sign can be seen easily.

When the sample or wastes are mailed, the sender must comply with Postal Code Article 8, No. 3, International Postal Code Articles 68 and 69, and the Universal Postal Convention 119 and 120.

3) The chief of the experiment must keep records of the recombinant's name, volume, and name of the recipient (the name of an experiment chief or an establishment) every time a recombinant is mailed or transported to an outside destination. However, when a P2 or lower confinement level recombinant is involved, an experiment record can replace this.

Appendix Section 10. Education and Training of Research Personnel (A matter related to Chapter 3, Section 1)

The chief of the experiment or the heads of universities or other research establishments are required to give an education and training to researchers involved, before the start of an experiment, about:

1. Microorganism handling techniques suitable for the degree of danger involved.

2. Techniques to realize required physical confinement levels.

3. Techniques to realize required biological confinement levels.

4. The danger involved in an experiment to be conducted.

5. Measures to be taken in case of an accident. (In conducting a volume culturing experiment, particular attention must be paid to the sterilization of a recombinant through chemical treatment, in preparation for an accidental leak of a cultured recombinant fluid.)

Appendix Section 11. Health-Keeping Measures for Research Personnel (A matter related to Chapter 3, Section 2)

The regular medical checkups and other measures that the heads of universities and other research establishments must take to keep the health of the researchers are as follows.

1. After a medical checkup before the start of an experiment, a regular medical examination must be given to researchers at least once every year. An ordinary medical examination conducted at those research institutes can be substituted for such an examination.

2. When an experiment requires the researcher to handle microorganisms having a pathogenicity, a preventive measure against the infection must be considered before start of the experiment, and when the need arises, antibiotics, vaccine, and blood serum must be kept in stock ready for use. In addition, after an experiment is started, an extra medical checkup must be given regularly at least once every six months.

3. When an experiment is conducted at the experiment zone where physical confinement level is P3 or higher, serum must be taken from the researchers before start of the experiment, and it must be preserved for two years after the end of the experiment.

4. When an infection of a lab researcher is suspected, he must be given an immediate medical checkup; if infection is confirmed, proper measures against the infection must be taken.

5. A prompt investigation must be launched when a researcher engaged in an experiment experiences or believes to have experienced any of the following incidents. Based on the investigation results, proper countermeasures must be taken.

1) When he accidentally swallows or inhales a recombinant.

2) When his skin is contaminated by a recombinant.

3) When he is in the lab or experiment zone when a serious contamination with a recombinant occurred in these experiment quarters.

4) When he begins to complain of ill health or when he falls seriously ill which would take him a long time to recover.

Appendix Section 12. Measures Required To Ensure Safety in Quasirecombinant DNA Experiments (A matter related to Chapter 2, Section 2, 1-2)-a) and Chapter 5)

A quasirecombinant DNA experiment is a recombinant DNA experiment involving the use of either a solid animal-originated sample or a plant.

I. Experiment Using Solid Animal-Originated Samples

1. "Experiment using solid animal-originated samples" refers to an experiment that involves the introduction of the molecules of a hetero-DNA into such samples as a fertilized egg, an embryo, a fetus, or an adult body to produce a new solid animal entity. The experiment also involves inoculating a solid animal entity with the molecules of a recombinant DNA or a recombinant. However, it excludes experiments (including the experiments in which no solid entities are generated) involving the introduction of the molecules or a hetero-DNA into a human body (including a fertilized egg) and experiments involving the inoculation of a human body (including a fertilized egg) with a recombinant or the molecules of a hetero-DNA.

In conducting a quasirecombinant DNA experiment, it is required to observe "the law for the protection and management of animals" (No. 105 law, 1973), "the standard for raising and keeping animals" (Prime Minister's Office notification No. 6, 1980), "the notice about the experiments involving the use of animals at universities and other research institutes" (Notice No. 141 by the head of the Education Ministry's Science and International Affairs Bureau, 26 May 1987), and other related regulations, and when the need arises, take appropriate measures under these guidelines.

2. In conducting an experiment involving the use of a solid animal sample, the research establishment's permission must be obtained before start of the experiment. However, an education minister's permission must be obtained when conducting any of the following experiments.

1) An experiment involving the use of any of the molecules of a hetero-DNA, the molecules of a recombinant DNA, or a recombinant that were created using those procaryote or lower eucaryote that have been found to have a pathogenicity, as the donor.

2) An experiment involving the use of any of the molecules of a hetero-DNA, the molecules of a recombinant DNA, or a recombinant that were created using any of those eucaryote viruses listed in Appendix Table 4-(1) and those not listed in Appendix Table 4 as the donor or vector.

3) An experiment involving the use of a primate.

4) An experiment in which the offspring obtained as a result of a recombinant experiment using a solid animal entity are put under management that is different from the management given to the first-generation entity.

5) An experiment involving the use of solid animal entities at a designated place outside a lab.

3. In an experiment involving the use of solid animal samples, the following safety measures must be taken.

1) A proper physical confinement level must be adopted by taking into consideration the characteristics of the molecules of the hetero-DNA, the molecules of the recombinant DNA, the recombinant, and the solid animal samples to be used.

When using any of the molecules of a hetero-DNA, the molecules of a recombinant DNA, or a recombinant that has a possibility of producing infectious viruses, a physical confinement level equivalent to the physical confinement level mandated in the experiment for producing and multiplying these molecules and recombinant must be exercised.

A sign indicating the physical confinement level in force must be posted at the entrance to the lab.

2) The entrance and exit doors, the air intake and exhaust ducts, and the water drainage pipes at the lab must be equipped with a facility to prevent escape of the animals involved in the experiment. The facility must be designed to take into account the habits of the animals involved.

3) Animals involved in an experiment should be treated independently as much as possible. When this is difficult, particularly in handling fish and insects, they should be managed by rearing group.

4) When rearing the offspring of first-generation animals, the same treatment as given to the first-generation animals should be given.

5) Measures should be taken to prevent people having nothing to do with the on-going experiment from entering the lab without permission.

6) The wastes of those animals should be incinerated and the water used to raise them should be sterilized, if necessary.

7) After finishing an experiment, those animal samples used in the experiment should be disposed after sterilization or burning.

8) The type of the hetero-DNA molecules, recombinant DNA molecules, and the recombinant used in an experiment must be recorded, and the record must be preserved.

9) When an animal is removed from the experiment zone, it must be done so by being contained in a sturdy, double-walled cage to prevent it from escaping. The cage should carry a sign on the surface where it can be easily seen, identifying the content.

4. When animals or their offspring being used in an experiment are moved from a university to another university or research establishment for use in a similar experiment, permission for the movement must be obtained from the heads of both establishments. This also applies to a case in which such movement is made to allow a researcher at one university to continue his research by changing the place of experiment to another university or research establishment. However, when moving the animals or their offspring that were produced in an education minister-permitted experiment, permission of the education minister must also be obtained.

5. The requirements described so far in this section do not apply to experiments involving the use of the hetero-DNA carrying animals that were recognized by the education minister as being "stable and safe" animals.

II. Experiments Using Plants

1. "Experiment using plants" refers to an experiment involving the introduction of the molecules of a hetero-DNA and an experiment involving the inoculation of plants with the molecules of a recombinant DNA or a recombinant (including the tissues containing a recombinant, organs, or other solid samples). The term "plant" comprises parts of a plant, including seeds, spores, and pollen; cultured and differentiated organs; and the cells and tissues that are cultured to promote a differentiation of an organ. The term "inoculation" includes infection, parasite, and symbiose. In conducting an experiment using plants, the measures stipulated in the guidelines must be implemented.

2. In conducting an experiment, permission must be obtained from the research establishment. However, an education minister's permission must be obtained when conducting any of the following experiments.

1) An experiment involving the use of any of the hetero-DNA molecules, recombinant DNA molecules, or a recombinant that were created using procaryote or lower eucaryote found to have a pathogenicity as the donor.

2) An experiment involving the use of any of the hetero-DNA molecules, recombinant DNA molecules, or the recombinants that were created using any of the eucaryote viruses listed in Appendix Table 4-(1) or not listed in Appendix Table 4 as the donor or vector.

3) An experiment involving a way of management of the offspring that is different from that given to the first-generation parent plant.

4) An experiment involving cultivation at specified places outside lab, including a culture or multiplication room, a greenhouse, a mesh room, or an isolated cultivation field.

3. In an experiment using plants, the following standards must be met to ensure experiment safety.

1) When culturing a sample using such closed-type culturing containers as test tubes and flasks:

a) The level of physical confinement adopted should be decided by considering the characteristics of hetero-DNA molecules introduced, recombinant DNA molecules, and a recombinant used for inoculation, and the plant used. However, when using any of the hetero-DNA molecules, recombinant DNA molecules, or the recombinant that has a possibility of producing infectious viruses, a physical confinement level equivalent to one mandated in the experiment for creation and multiplication of them must be adopted.

b) People who have nothing to do with an on-going experiment must not be allowed into the lab or experiment zone within the lab without permission.

c) When culturing the offspring of a plant used in an experiment, the same sample management accorded to the first-generation parent plant must be given to the offspring.

d) After finishing an experiment, the plant and the microorganism and the animal that were used, together with the plant, must either be sterilized or incinerated. The test tubes and flasks used in the experiment must be sterilized.

e) When using a microorganism together with an animal, a safety measure suitable to such an experiment condition must be taken by taking their characteristics into consideration.

f) In an experiment for producing spores, pollen, and seeds, care must be exercised to prevent their escape out of the lab through scattering or by being carried by insects.

g) Records must be kept about the hetero-DNA molecules introduced and the recombinant DNA molecules and the recombinant inoculated, and the record must be preserved.

2) When conducting an experiment using an open-type culturing equipment, such as a flowerpot:

a) The level of physical confinement must be decided by considering the characteristics of the hetero-DNA molecules introduced, the recombinant DNA molecules, and the recombinant used for

inoculation, and the plant used. However, when using any of the hetero-DNA molecules, recombinant DNA molecules, or the recombinant having a possibility of producing infectious viruses, a physical confinement level equivalent to one mandated in the experiment for creation and multiplication of them must be adopted.

b) People who have nothing to do with an on-going experiment must not be admitted into the lab or the experiment zone within the lab without permission.

c) When culturing the offspring of a plant used in an experiment, the same sample management accorded to the first-generation parent plant must be given to the offspring.

d) After finishing an experiment, the plant used and the microorganism and animal samples used, together with the plant, must either be sterilized or incinerated. The pots and soil used in the experiment and the waste water resulting from the experiment must be sterilized.

e) In an experiment for producing spores, pollen, and seeds, proper measures, including wrapping the flowers with paper bags, must be taken to prevent scattering of the seeds before ripening.

f) Insects and other creatures that have a possibility of carrying spore, pollen, and seeds out of the lab must be eliminated from the lab.

g) To prevent escape of spore, pollen, and seeds out of the lab, researchers must put on a designated uniform when they enter the lab, and take it off and wash their hands when they leave the lab.

h) When using a microorganism and an animal together with a plant, the plant should be isolated from them by being put in a growth cabinet, if necessary.

i) Detailed records of the hetero-DNA molecules introduced, and the recombinant DNA molecules and the recombinant used for inoculation must be kept, and the record must be preserved.

4. Preservation of the plant used in the experiment must be done according to the guideline stipulations about preservation of recombinants.

5. When offering a plant being used in an experiment at one university to another university or research establishment, permission must be obtained from the heads of the two establishments. This also applies when moving the plant from one establishment to another to allow a researcher to continue his research by changing the place of research. However, when having a plant that was created in an education minister-permitted experiment donated from an outside establishment, permission of the education minister is additionally required.

6. The requirements described so far in this section do not apply to the hetero-DNA molecule-carrying plants that were recognized by the education minister as being "stable and safe."

Appendix Tables

Appendix Table 1. Standards for Safety Cabinets and HEPA Filters

Class I	
Application field	Used in an experiment handling microorganisms or pathogens having a low or a middle-level danger and requiring no purified air in the experiment zone.
Structural specification	Required to have an air intake opening in the front and an exhaust air outlet. The air current from the front opening must prevent the contaminated aerosol produced inside the cabinet from being exhausted without treatment. The air inside the cabinet must be exhausted out of the cabinet after filtering using an HEPA filter. The average intake air speed (exhausted air volume/area of the front opening) must have a value higher than 0.40 m/s.

Appendix Table 1. Standards for Safety Cabinets and HEPA Filters

Class II	
Application field	Used in an experiment that involves handling of the microorganisms or pathogens having a low to medium level of danger and requiring purification of the air in the working space. Two types of safety cabinet are available: Type A is designed for use in ordinary biological experiments; Type B for use in handling a small amount of harmful and dangerous chemicals, radioactive materials, and gaseous substances, which cannot be removed with HEPA filter efficiently.
Structure	Required to have an air intake opening in the front and an air exhaust outlet. The air current taken in through the front opening must prevent escape of the contaminated aerosol from the cabinet. The air taken through the opening must be supplied to the working space in a streamlined flow after being purified using an HEPA filter. Exhaust of the air must be made through an HEPA filter out of the cabinet. In a Type A cabinet, the use of a structure that calls for a positive-pressure contamination plenum joined to the outer wall of the [cabinet] should be avoided. When using a Type B cabinet, the air from the working space must be exhausted out of the experiment building through an exhaust duct connected to the cabinet.
Specification	**Airtightness:** When the inside of the cabinet is pressurized with air equivalent to 50 mm in height of a water column, the drop of the pressure must be confined to less than 10 percent 30 minutes after the pressurization stops. When soapy water or a leak detection fluid is applied to all the welded portions and the places where the safety cabinet parts pass through the walls of the cabinet, no formation of bubbles—indicating a leak—must be observed. (In the cabinet type in which the positive-pressure plenum is joined to the outer wall of the cabinet, the leak volume of halide gas introduced into the cabinet must be lower than $5x10^{-7}$ cc/sec.)
	Test for Safety of Researcher: When a safety test is conducted by spraying 5 to $10x10^{8}$ cfu (colony forming unit) of bacillus subtilis spores, the total number of the colony of the bacillus caught by four units of impingers must be below 10. The number of colonies caught by a slit sampler after a period ranging from five to 15 minutes after the start of the test must be fewer than five each time the test is made. In three tests conducted consecutively, the number of colonies counted must be below five, respectively.
	Sample Protection Test: In a sample protection test conducted by spraying bacillus subtilis spores with the density ranging from 5 to $10x10^{6}$ cfu, the number of colonies of the bacillus caught by an array of agar medium-filled 10 cm-diameter laboratory dishes must be fewer than five in total every time when the test is conducted three times consecutively. (In the test, as many numbers of agar medium-filled laboratory dishes as possible should be laid on the floor. This requirement applies whenever a reference is made to a term "agar medium plates" in the following of this table.)
	Test To Prevent Mutual Contamination of Test Samples: In a test to check on the mutual contamination by spraying bacillus subtilis spores with the density ranging from 5 to $10x10^{4}$ cfu, the number of the colonies of the bacillus at points 355 mm or more away from the left-side or right-side wall of the cabinet on the agar medium plates is required to be fewer than two in total. This level of the colony must be attained every time the test is conducted three times from the left-hand side and the same number of test from the right-hand side inside the cabinet.
	Blow-Out Speed: The speed of the air blowing out of the safety cabinet, measured at points inside a 15 cm lattice window at a given moment, must be within +/- 20 percent of the mean speed. In a safety cabinet, which is designed to produce a gradient in the speed of blow-out air, the measurement and calculations must be made within the lattice windows designated by the cabinet designer.
	Air Speed at the Intake Opening: The air speed at the air intake opening of the cabinet must have a mean value higher than 0.40 m/s (higher than 0.50 m/s in a Type B cabinet).
	Air Blower: The air blower must keep a loss of moving air volume within 25 percent without controlling the revolution of the blower motor when the pressure loss at the cabinet filter increases by 20 percent.
	Direction of Air Current: The direction of air flow within the cabinet can be seen by observing the moving direction of the smoke from a smoke generator. When the working space inside the cabinet is scanned between the left- and right-side walls at a height of 100 mm +/- 10 mm from the bottom of the front panel at the point where the downward streamlined air current is diverted both to the air ventilation openings in the front and the rear, and at a height of 150 mm +/- 20 mm from the bottom of the front panel with a distance ranging from 20 to 30 mm from the panel within the cabinet, the smoke must flow smoothly downward. It must be ensured that there is no place where the smoke lingers or flows upward. There must be no place where the smoke escapes out of the cabinet. When scanned across the front opening at a point 30 to 40 mm away from the opening outside the cabinet, the smoke that entered into the cabinet through the opening must not be detected as leaking from the cabinet. The smoke also must not be detected as leaking into the working space.
	Temperature Rise: The difference between the room temperature and the temperature inside the cabinet must be maintained within 8°C after four hours of continuous operation.
	Noise Level: The noise level must be kept below 67 dBA.
	Illumination: The average brightness must be kept between 800 and 1,200 lux.
	Vibration: The displacement in the work table due to vibration in three orthogonal directions must be kept below 5 μm RMS.
	Fluid Receiving Pan: The fluid receiving pan must have a capacity larger than 4 liters and have a construction allowing easy cleaning.

Appendix Table 1. Standards for Safety Cabinets and HEPA Filters

Consideration on cleaning and sterilization	The sections that are exposed to the fluids used in an experiment directly or indirectly must have a surface condition that allows cleaning of the surface without using any cleaning tool. The working table and the corner sections in the working space must be cleaned and sterilized carefully. The cabinets are required to have a construction that allows conducting a formaldehyde sterilization without moving them. Also required to have a construction allowing the front opening and the exhaust outlet to be sealed using metal plates, plastic sheets, or adhesive tapes. To make cleaning easier, the gap between the lab floor and the bottom of the cabinet must be wider than 80 mm, or an adhesive sealing must be applied to the floor or the cabinet base.
Performance check	There is a possibility of trouble occurring in the cabinet not long after the start of its use, such as a clogged HEPA filter, which could directly affect the operation of the cabinet. Considering this, it is recommended that a performance check be conducted immediately after installation of the cabinet, and, after that, once a year every year.
Class III	
Application field	Used in experiment handling the microorganisms or pathogens having a high level of danger.
Structural specification	In a closed-type cabinet, intake of the air must be made through an HEPA filter and exhaust of the air through a two-stage HEPA filter. Otherwise, the exhaust gas must be passed through an incineration sterilization system before it is exhausted outdoors. The working space must be kept at a pressure lower than that in the lab, with the difference equivalent to 15 mm or higher in water column height. Pairs of protective gloves and a high-pressure sterilizer or a sterilization fluid-filled vessel for sterilization of samples and equipment used inside the cabinet must be provided.
HEPA Filter for Use With Safety Cabinet	
Capability and other factors having to be considered	When a permeability test of HEPA filter is conducted by loading aerosol to the primary side of it, the ratio of the density of the applied aerosol in the secondary side against the primary-side density at a given segment of the filter must not exceed 0.01 percent. When a scanning test is conducted by mounting the filter on the cabinet under a near constant-speed suction condition, using a relative density measurement apparatus or a particle counter having a suction capacity of 28.3 liters per minute, it is required that the permeability of aerosol near 0.3 μm range [in aerosol particle size] does not exceed 0.01 percent. Required to use an aluminum separator. The installment of a pressure gauge indicating the change in the pressure lose in the HEPA filter is recommended.

Appendix Table 2. Education Minister-Permitted Host-Vector Systems and Their Biological Confinement Levels

1. B1 Level Host-Vector Systems

1) EK1

A host-vector system in which E. coli K12 or its derivative is used as the host and plasmid or bacteriophage is used as the vector. E. coli K12 is a species of the E. coli that is commonly known both genetically and physiologically as a virus having no toxicity and a low survivability under natural environment conditions. Plasmid and bacteriophage have no conjugation capability and thus are not transferable to other kinds of bacteria. (In the host-vector system, the host must not contain plasmid or bacteriophage having a conjugation capability.)

2) BS1

A host-vector system in which the host is a strain of the derivative of bacillus subtilis marburg 168 having mutational multiple nutrient-demanding characteristics vis-a-vis amino acids or nucleic acid base, and the vector is either a plasmid (having no transferability to other cells as a result of a conjugation) or a bacteriophage created using bacillus subtilis as their host.

3) SC1

A host-vector system in which the host is the lab-preserved strain of yeast S. cerevisiae and the vector is either of the plasmid or minichromosome of yeast S. cerevisiae or their derivatives.

4) Cultured Cells of Animals or Plants

A host-vector system using cultured cells of animals or plants as the host (excluding the case when infectious viruses are produced).

2. B2 Level Host-Vector System

EK2

A host-vector system in which the host is either of those listed in the following table which, satisfying the condition required for EK1, has a very low survivability due to the genetic defects except under a special culturing condition; the vector is any of those listed in the right column of the following table which, having particularly high dependency on the host, has a very low transferability to other living cells. In the host-vector system, the number of the living cells carrying DNA recombinant molecules declines to fewer than 1/100 million, except when being put under a special culturing environment 24 hours [after formation of the system].

Host	Vector
χ1776	pSC101
	pCR1
	pMB9
	pBR313
	pBR322
	pBR325
	pDH24
	pGL101
	YIp1
	YEp2
	YEp4
	YIp5
	YEp6
	YRp7
	YEp20
	YEp24
	YEp24
	YIp26
	YIp27
	YIp28
	YIp29
	YIp30
	YIp31
	YIp32
	YIp33
	pKY2662
	pKY2738
	pKY2800
DP50supF	λWES.λB
	λgtALOλB
	Charon21A
E. coliK12	λtυJZ-B
DP50	Charon3A
DP50supF	Charon4A
	Charon16A
	Charon23A
	Charon24A

Appendix Table 3. Classification of Procaryote (Including Rickettia and Chlamydia) and Lower Eucaryote as DNA Donor by Their Safety Level

(1)

Brucella abortus
Brucella canis
Brucella melitensis
Brucella ovis
Brucella suis
Coccidioides immitis
Coxiella burnetii
Francisella tularensis
Histoplasma capsulatum
Histoplasma duboisii
Histoplasma farciminosum
Mycobacterium avium/intracellulare
Mycobacterium bovis
Mycobacterium fortuitum
Mycobacterium kansasii
Mycobacterium szulgai
Mycobacterium tuberculosis
Mycoplasma mycoides
Paracoccidioides braziliensis
Pseudomonas mallei
Pseudomonas pseudomallei
Rickettsia akari
Rickettsia australis
Rickettsia canada
Rickettsia conorii
Rickettsia montana
Rickettsia parkeri
Rickettsia prowazekii
Rickettsia rhipicephali
Rickettsia rickettsii
Rickettsia sibirica
Rickettsia tsutsugamushi
Rickettsia typhi
Rochalimaea quintana
Rochalimaea vinsonii
Yersinia pestis

(2)

Bacillus anthracis
Blastomyces dermatitidis
Bordetella pertussis
Borrelia All species
Calymmatobacterium granulomatis
Campylobacter fetus
    subspecies fetus
    subspecies venerealis
Campylobacter coli
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Clostridium botulimon
Clostridium chauvoei
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi
Clostridium perfringens
Clostridium speticum
Clostridium tetani
Corynebacterium bovis
Corynebacterium diphtheriae
Corynebacterium equi
Corynebacterium ovis/pseudotuberculosis
Corynebacterium ulcerans
Corynebacterium haemolyticum
Corynebacterium pyogenes
Corynebacterium renale
Cryptococcus neoformans

Erysipelothrix rhusiopathiae
Klebsiella pneumoniae
Legionella All Species
Leptospira interrogans Total Serum type
Listeria monocytogenes
Mycobacterium africanum
Mycobacterium bovis
Mycobacterium bovis (BCG Strain)
Mycobacterium chelonei
Mycobacterium leprae
Mycobacterium marinum
Mycobacterium scrofulaceum
Mycobacterium simiae
Mycobacterium ulcerans
Mycobacterium xenopi
Mycoplasma pneumoniae
Naegleria fowleri
Neisseria gonorrhoeae
Neisseria meningitidis
Nocardia asteroides
Nocardia brasiliensis
Nocardia otitidiscavariarum
Nocardia farnicica
Pasteurella haemolytica
Pasteurella multocida
Pasteurella pneumotropica
Salmonella Total serum type
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum
Treponema pallidum
Treponema pertenue
Vibrio cholerae
Vibrio vulnificus
Yersinia enterocolitica
Yersinia psuedotuberculosis

Appendix Table 4. Classification of Viruses and Full-Length Viroid Genomes Belonging to Eucaryote (Excluding Ones Belonging to Lower Eucaryote) as DNA Donor by Safety Level

(1)

African horse sickness virus
African swine fever virus
Colorado tick fever virus
Congo hemorrhagic fever virus
Ebola virus
Foot-and-mouth disease virus
Herpes B virus
Junin virus
Kyasanur forest disease virus
Lassa fever virus
Machupo virus
Marburg disease virus
Rift Valley Fever virus
Rinderpest virus
Russian spring-summer encephalitis virus
Tick-borne encephalitis virus
Variola major virus
Variola minor virus
Venezuelan encephalitis virus
Yellow fever virus

(2)

California encephalitis virus
Chikungunya virus
Creutzfeldt-Jakob disease agent
Hemorrhagic fever with renal syndrom virus
Herpes ateles virus
Herpes saimiri virus
Hog cholera virus
HIV (Human immunodeficiency virus)*1
Scrapie agent
SIV (Simian immunodeficiency virus)*2
Japanese encephalitis virus
La Crosse virus
LCM virus
Monkeypox virus
Murray valley encephalitis virus
O'nyong-nyong virus
Powassan virus
Rabies (street) virus
St. Louis encephalitis virus
Tacaribe virus
Vesicular stomatitis virus
West Nile virus

*1 HTLV-III = HIV-1, HTLV-IV = HIV-2, LAV-1 = HIV-1, LAV-2 = HIV-2
*2 STLV-III is included in SIV.

(3)

Adenovirus (human)
Avian reticuloendotheliosis virus
BIV (Bovine immunodeficiency virus)
Batai virus
Cowpox virus
Coxsackie virus (A, B)
Cytomegalovirus (human, animal)
Dengue virus (1-4)
Eastern equine encephalitis virus
EB virus
Echovirus (1-34)
Ectromelia virus
EMC (Encephalomyocarditis virus)
Enterovirus (68-71)
Equine infectious anemia virus
Equine rhinopneumonitis virus
FIV (Feline immunodeficiency virus)
Hepatitis A virus
Hepatitis B virus
Hepatitis (non A non B) virus

Herpes simplex virus (1, 2)
HVJ (Sendai virus)
Infectious bovine rhinotracheitis virus (bovine herpes virus)
Influenza virus (human)
Mammalian retrovirus *3
    HTLV (Human T-cell leukemia virus) *4
    STLV (Simian T-cell leukemia virus) *5
    Other Mammalian lentivirus
Measles virus
Molluscum contagiosum virus
Mouse hepatitis virus
Mumps virus
NDV
Papovavirus
    human: BKvirus, JCvirus, human papillomavirus
    non-human: Bovine papilloma, lymphotropic papovavirus, polyoma virus, SV40, other non-human papillomavirus, other non-human polyomavirus
Parainfluenza virus (1-4)
Pichinde virus
Poliovirus (1-3)
Pseudorabies virus (Swine herpesvirus)
Rabies (fixed, attenuated) virus
Rhinovirus
Rinderpest virus (vaccine strain)
Rotavirus
Rubella virus
Semliki Forest virus
Simian herpes virus (Herpes B virus, Herpes Ateles virus, Herpes samiri virus excluded)
Tanapox virus
Vaccinia virus
Varicella virus
Western equine encephalitis virus
Yaba virus

*3 HIV, SIV, HTLV, STLV excluded.
*4 HTLV-III, HTLV-IV excluded.
*5 STLV-III excluded.

(4)

Adenovirus (avian, canine, bovine, porcine, murine)
Aino virus
Akabane virus
Avian encephalomyelitis virus (Picornaviridae)
Avian enterovirus
Avian poxvirus
Avian retrovirus (Avian reticuloendotheliosis virus excluded)
Bluetongue virus
Bovine viral diarrhea virus (Togaviridae)
Bunyamwera virus
Calicivirus (human, animal)
Canine distemper virus
Canine herpesvirus
Coronavirus (Mammalian, Avian)
Duck hepatitis virus
Enterovirus (Swine, Bovine)
Equine arteritis virus (Togaviridae)
Feline rhinotracheitis virus (Feline herpesvirus)
Fish viruses (limited to IPN, IHN, EVA, EVE, LV)
Getah virus
Infectious bursal disese virus (Birnaviridae)
Infectious laryngotracheitis virus (Herpesviridae)
Influenza virus (avian, equine, swine)
Insect viruses (limited to species of those that are not infectious to vertebrate animals, including arbovirus)
Kilham rat virus
Lactic dehydrogenase Virus
Langat virus
Live virus vaccine strains (excluding rinderpest vaccine strain)
Lucke virus
Mouse encephalomyelitis virus (Picornaviridae)
Marek's disease virus (including herpes virus of turkey)
Parvovirus (human, animal)
Plant viruses
Pneumonia virus of mice (PVM) (Paramyxoviridae)
Poikilothemal vertebrate retrovirus
Reovirus (1-3)
Shope fibroma virus
Simbu virus
Sindbis virus
Swinepox virus
Viroids

Appendix Table 5. Host-Vector Systems Ascertained To Have a High Level of Safety When Particular Types of DNA Donors Are Used

Host-vector systems	DNA donors	Mandated physical confinement level
The host-vector system in which the host is any of those listed below and the vector is either plasmid or bacteriophage		
Acetobacter aceti		
Acetobacter pasteurianus		
Bacillus amyloliquefaciens		
Bacillus brevis		
Bacillus stearothermophilus		
O Brevibacterium flavum		
O Brevibacterium lactofermentum		
Corynebacterium glutamicum		
O Cornyebacterium herculis	Among procaryote, lower eucaryote, rickettia and chlamydia, those that are not listed in Appendix Table 3 (excluding those that have been found to have a pathogenicity)	P1
Corynebacterium ammoniagenes		
Gluconobacter oxydans		
Lactobacillus helvetics		
Pseudomonus putida		
Streptococcus cremoris		
Streptococcus lactis		
Streptococcus thermophilus		
O Streptomyces kasugaensis		
O Streptomyces lividans		
The host-vector system in which the host is any of those lower eucaryote listed below and the vector is either plasmid or minichromosome		
Acremonium chrysogenum		
Aspergillus oryzae		
Aspergillus sojae		
Neurospora crassa		
Pichia pastoris		
Saccharomycopis lipolytica		
Schizosaccharomyces pombe		
Trichoderma viride		
Zygosaccharomyces rouxii		

O mark denotes commonly used names.

Appendix Table 6. Special Cases of Using Viruses Infectious to Humans and Multiplying Within the Human Body as the Vector

Adenovirus (human)
Cowpox virus
Cytomegalovirus (human)
EB virus
Herpes simplex virus (1,2)
HTLV*
Mammalian lentivirus (other than human)
Mammalian rentovirus (other than human)
Molluscum contagiosum virus
Papovavirus
STLV
Vaccinia virus
Varicella virus
Yaba virus
Live virus vaccine strains (excluding rinderpest vaccine strain)

*See the footnote in Appendix Table 4.